Plasma Carotenoids and Tocopherols and Risk of Myocardial Infarction in a Low-Risk Population of US Male Physicians

A. Elisabeth Hak, MD, PhD; Meir J. Stampfer, MD, DrPH; Hannia Campos, PhD; Howard D. Sesso, DSc; J. Michael Gaziano, MD; Walter Willett MD, DrPH; Jing Ma, MD, PhD

Background—Increased intake of carotenoids and vitamin E may protect against myocardial infarction (MI). However, prospective data on blood levels of carotenoids other than β-carotene and vitamin E (tocopherol) and risk of MI are sparse.

Methods and Results—We conducted a prospective, nested case-control analysis among male physicians without prior history of cardiovascular disease who were followed for up to 13 years in the Physicians’ Health Study. Samples from 531 physicians diagnosed with MI were analyzed together with samples from paired control subjects, matched for age and smoking, for 5 major carotenoids (α- and β-carotene, β-cryptoxanthin, lutein, and lycopene), retinol, and α- and γ-tocopherol. Overall, we found no evidence for a protective effect against MI for higher baseline plasma levels of retinol or any of the carotenoids measured. Among current and former smokers but not among never-smokers, higher baseline plasma levels of β-carotene tended to be associated with lower risk (P for interaction=0.02). Men with higher plasma levels of γ-tocopherol tended to have an increased risk of MI (P for trend=0.01).

Conclusions—These prospective data do not support an overall protective relation between plasma carotenoids or tocopherols and future MI risk among men without a history of prior cardiovascular disease. (Circulation. 2003;108:802-807.)

Key Words: antioxidants ▪ cardiovascular diseases ▪ plasma ▪ myocardial infarction

The “oxidative modification hypothesis” of coronary heart disease (CHD)1 proposes that intake of antioxidants, such as carotenoids and vitamin E, protects against atherogenesis by blocking oxidation of low-density lipoprotein cholesterol.2 Additionally, antioxidants may favorably influence plaque stability, vasomotor function, and tendency for thrombosis.3

Carotenoids are plant-derived, fat-soluble pigments efficient in quenching singlet oxygen and free radicals.4 Prospective observational studies have found inverse associations between dietary intakes and blood levels of carotenoids (primarily β-carotene) and risk of CHD.5 Null results from randomized trials of β-carotene supplementation6–8 suggest, however, that other nutrients in β-carotene–rich foods may be responsible for the apparent benefit. This was further suggested by recent data showing a protective effect of lutein in early atherogenesis.9 Few studies have examined the prospective association between carotenoids other than β-carotene and risk of CHD.10,11

Intake of vitamin E (tocopherol) has been associated with lower risk of CHD in observational studies of healthy individuals.12–14 However, large trials among subjects with established CHD or at high risk of CHD found no benefit.6,15,16 The one large prospective study on blood levels of vitamin E and CHD, which found no association, was also conducted in a high-risk population.17 Conceivably, vitamin E may be effective in inhibiting earlier stages of atherosclerosis.18

We prospectively examined the associations between plasma levels of 5 major carotenoids (α- and β-carotene, β-cryptoxanthin, lutein, and lycopene), retinol, and tocopherols with myocardial infarction (MI) in a low-risk population of male physicians.

Methods

Study Population, Dietary Intake, and Collection of Blood Samples

The methods of the Physicians’ Health Study have been described in detail elsewhere.7 Briefly, 22,071 male physicians, age 40 to 84 years in 1982, with no history of cardiovascular disease or cancer, were assigned randomly in a factorial design to receive aspirin (325 mg), β-carotene (50 mg), or placebo. Informed consent was obtained...
from all participants, and the research protocol was approved by the institutional review board at Brigham and Women’s Hospital in Boston. At baseline, the physicians completed questions on their health status and risk factors for cardiovascular disease. Dietary intakes of selected types of fruit and vegetables were ascertained by two abbreviated, semiquantitative food frequency questionnaires. Seven response categories ranged from rarely/never to 2 or more per day. Reproducibility and validity of the questionnaire items on fruit and vegetable intake were found to be reasonable in a study of 127 male health professionals 45 to 70 years of age. Before random assignment, 14,916 (68%) participants provided baseline blood samples in EDTA tubes, which were processed and stored at −80°C. More than 70% of the samples were received between September and November 1982. During storage, precautions were taken so that no specimen thawed or warmed substantially. Degradation of carotenoids, retinol, and tocopherols was nondetectable in plasma stored at −70°C for up to 51.5 months, and β-carotene, retinol, and α-tocopherol are stable for at least 15 years at temperatures −70°C. Intraperson correlation coefficients based on repeated blood samples taken from 22 control subjects at baseline and after 5 years of follow-up were reasonable for carotenoids and tocopherols, ranging from 0.52 to 0.85 (all probability values ≤0.01).

### End Point Definition and Selection of Control Subjects

Six months after random assignment and annually thereafter, participants completed mailed questionnaires that inquired about compliance with their assigned treatment and the occurrence of end points of interest, including MI. Nonresponders to the questionnaires were telephoned. Deaths were usually reported by family members or postal authorities. Morbidity and mortality follow-up is >99% complete.

Medical records were reviewed by the end point committee without knowledge of treatment assignment. All cases of MI included in this analysis met World Health Organization criteria, which require symptoms plus either enzyme elevations or diagnostic ECG changes. For fatal cases, diagnoses were based on either autopsy or confirmation through records that death was due to coronary heart disease (International Classification of Diseases, Ninth Revision [ICD-9] codes 411–414). Silent infarcts discovered on routine examination were not included because they could not be

### TABLE 1. Baseline Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Myocardial Infarction Cases</th>
<th>Control Subjects</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58.1±8.6</td>
<td>57.9±8.5</td>
<td>Matching factor</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td></td>
<td></td>
<td>Matching factor</td>
</tr>
<tr>
<td>Current smoker</td>
<td>14.7</td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>40.9</td>
<td>40.9</td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>44.4</td>
<td>44.4</td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.5±3.4</td>
<td>24.9±2.9</td>
<td>0.004</td>
</tr>
<tr>
<td>Lipoproteins, mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>228.3±41.5</td>
<td>218.0±39.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>44.6±12.4</td>
<td>49.2±13.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides†</td>
<td>180.1±117.2</td>
<td>146.8±95.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Medical history, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension‡</td>
<td>39.6</td>
<td>25.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6.3</td>
<td>2.7</td>
<td>0.005</td>
</tr>
<tr>
<td>Parental history of myocardial infarction before 60 y, %</td>
<td>17.1</td>
<td>13.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Vigorous exercise less than once weekly, %§</td>
<td>36.3</td>
<td>26.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Alcohol intake, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 1 weekly</td>
<td>29.5</td>
<td>27.6</td>
<td></td>
</tr>
<tr>
<td>1 to 6 drinks weekly</td>
<td>48.2</td>
<td>45.2</td>
<td></td>
</tr>
<tr>
<td>Daily</td>
<td>22.3</td>
<td>27.2</td>
<td>0.18</td>
</tr>
<tr>
<td>Multiple vitamin use, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>65.2</td>
<td>63.5</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>15.5</td>
<td>15.1</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>19.3</td>
<td>21.4</td>
<td>0.70</td>
</tr>
<tr>
<td>β-Carotene assignment, %</td>
<td>46.7</td>
<td>50.5</td>
<td>0.22</td>
</tr>
<tr>
<td>Aspirin assignment, %</td>
<td>43.1</td>
<td>45.8</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Values are mean±SD or percentages.

*χ² statistic for categoric variables and paired t test for continuous variables.
†Data available for 369 cases and their matched control subjects.
‡Self-reported blood pressure was at least 140/90 mm Hg, or the subject was taking antihypertensive medication.
§Vigorous exercise was defined as “exercise vigorous enough to work up a sweat.” |
| (The aspirin arm of the trial was stopped after 5 years.)
assigned an accurate date. Sudden deaths in individuals with no history of coronary disease were not included unless coronary disease could be confirmed as the specific cause of death.

Each case was matched to one control subject who was free from MI at the time of diagnosis of MI in the case. Control subjects were randomly selected from participants who met the matching criteria of age (within 1 year of the case age), smoking habit (past, current, or never smoker), and time since random assignment in 6-month intervals.

**Laboratory Analyses**

Plasma levels of carotenoids, retinol, and tocopherols were measured as described previously. Briefly, each case and its matching control samples were assayed in the same batch by laboratory personnel blinded for case-control status. Carotenoids, retinol, and tocopherols were assayed by high-performance liquid chromatography. Mean intra-assay coefficients of variation based on blinded quality control samples ranged from 7.6% for ß-carotene to 11.9% for α-tocopherol. Total and HDL cholesterol levels and triglyceride concentrations were assayed as described previously.

**Statistical Analysis**

For baseline cardiovascular disease risk factors, means or proportions were calculated for men who had MI (cases) and control subjects. We assessed statistical significance by χ² tests for categorical variables and paired t tests for continuous variables.

For all analyses, we used weighted batch-specific values of plasma carotenoids, retinol, or tocopherols to account for differences in levels arising from using various batches to assay the large number of samples. Because each case and its matched control sample were assayed in the same batch, this procedure preserves the validity of our results.

The partial Spearman rank-correlation coefficient was used to test the multivariate-adjusted association between the baseline consumption (7 response categories) of a specific fruit or vegetable known to be predictive of the plasma carotenoid level and the measured plasma levels in control subjects. We also computed plasma levels of α- and γ-tocopherol according to baseline multivitamin use. Because the lipid-soluble carotenoids and tocopherols are carried in lipoproteins, we adjusted the correlation coefficients for total and HDL cholesterol. While computing the adjusted tocopherol levels, we additionally adjusted for triglycerides because of the strong association between lipid levels and triglycerides. We then performed conditional logistic regression analyses, based on the case-control matching. Adjusted estimates of risk were obtained with multivariate models that also controlled for potential confounding factors. Because multivitamin use was the major determinant of tocopherols, we did not adjust for multivitamin use in models regarding tocopherols. Tests for trend were performed by entering a variable representing the median antioxidant plasma levels for the quintiles of the distribution among the controls.

To assess whether results observed for plasma ß-carotene were affected by assignment to either ß-carotene or placebo, we fit unconditional logistic regression models within the active supplement and placebo groups and fit an unconditional logistic regression model with a multiplicative interaction term involving ß-carotene assignment and baseline plasma ß-carotene level. Using the same approach, we evaluated whether the associations for baseline ß-carotene or α-tocopherol levels were affected by smoking status (current, former, never).

All reported probability values are 2-tailed, and 95% confidence intervals were computed.

**Results**

Table 1 shows the baseline characteristics of the 531 subjects who had a first MI and their matched control subjects. The mean time from study enrollment to the occurrence of MI was 6.3 years (SD, ±3.5 years), with a maximum follow-up of 13.0 years. As expected, compared with control subjects, MI cases had a more adverse risk profile. During follow-up, 12 control subjects eventually had a myocardial infarction, all of whom were subsequently matched to new disease-free control subjects in our analyses.

The baseline plasma levels of carotenoids were modestly but significantly correlated with the reported consumption of specific fruits or vegetables known to be predictive of these levels, as shown by the partial correlation coefficients in Table 2. Baseline plasma levels of α-tocopherol were higher in multivitamin users compared with nonusers, whereas levels of γ-tocopherol were lower. Among control subjects, age- and lipoprotein-adjusted (total and HDL cholesterol and triglycerides) geometric mean values of α-tocopherol in multivitamin users and nonusers were 14 730 and 10 743 ng/mL, respectively, whereas corresponding levels of γ-tocopherol were 1371 and 1822 ng/mL (both probability values <0.001). Plasma levels of α-tocopherol and γ-tocopherol were modestly correlated (r=0.15, P<0.05).

As shown in Table 3, apart from a weak tendency toward lower risk for men with ß-carotene levels in the second up to fifth quintile, men with higher baseline plasma levels of carotenoids or retinol did not have a lower risk of subsequent MI. Men with higher baseline plasma levels of tocopherols had an increased risk of MI. Multivariate adjustment attenuated the associations for α-tocopherol, but the elevated risk for γ-tocopherol persisted (P for trend=0.01).

In subgroup analyses, we observed no material differences between baseline plasma ß-carotene levels and risk of MI according to random assignment to ß-carotene or placebo. However, among past and current smokers, men with higher baseline plasma levels of ß-carotene tended to have a lower risk of MI (multivariate-adjusted OR 5th versus 1st quintile, 0.55 (95% CI, 0.27 to 1.12) and 0.46 [CI, 0.10 to 2.06], respectively), whereas among never-smokers, we found no tendency toward a lower risk (OR, 0.96 [CI, 0.46 to 1.99], P-interaction=0.02). Results for α-tocopherol were not modified by smoking status (P-interaction=0.85).

**TABLE 2. Correlation Coefficients Between Plasma Levels of Carotenoids and Intake of Selected Fruits or Vegetables Among 531 Control Subjects**

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Fruit or Vegetable*</th>
<th>ρ†</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Carotene</td>
<td>Carrots</td>
<td>0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Carrots</td>
<td>0.14</td>
<td>0.001</td>
</tr>
<tr>
<td>β-Cryptoxanthin†‡</td>
<td>Orange juice</td>
<td>0.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lutein‡</td>
<td>Spinach, cooked</td>
<td>0.11</td>
<td>0.05</td>
</tr>
<tr>
<td>Lycopene‡</td>
<td>Tomato juice</td>
<td>0.13</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Selected fruits and vegetables were ascertained by 2 abbreviated questionnaires. Seven response categories ranged from rarely/never to 2 or more per day.

†Partial correlation coefficient adjusted for age, smoking (current, former, never), body mass index, levels of total and HDL cholesterol, alcohol consumption (7 categories), and multivitamin use (never, past, current). In n=257.
### Discussion

In this prospective, nested case-control study of generally well-nourished healthy male physicians without diagnosed cardiovascular disease at enrollment, we found no evidence for a protective effect against MI for higher baseline levels of plasma retinol or carotenoids, apart from a tendency toward lower risk among those with higher levels of \( \beta \)-carotene among current and former smokers. Higher plasma levels of \( \gamma \)-tocopherol were associated with an increased risk of MI.

These analyses were based on a single baseline measurement of carotenoids and tocopherols and may therefore not reflect levels over a longer period with complete accuracy. However, our baseline measurements were reasonably correlated with levels measured 5 years later. In addition, we observed reasonable correlations of plasma carotenoids with intake of specific fruits and vegetables, and plasma tocopherol levels differed between multivitamin users and nonusers.

Previous results from the Physicians’ Health Study showed that \( \beta \)-carotene supplementation does not affect MI risk. Although \( \beta \)-carotene supplementation increases \( \beta \)-carotene levels, it does not affect concentrations of other carotenoids or tocopherols. Hence, the possible inverse relation with \( \beta \)-carotene among smokers may reflect a benefit of some other nutrient(s) in foods rich in \( \beta \)-carotene. The plasma levels of tocopherols reflect mainly intake from low-dose multivitamin supplementation and therefore do not necessarily reflect potential effects of high-dose vitamin E supplements. A further consideration is that Physicians’ Health Study participants have low cardiovascular mortality rates, only 15% that of the general population of white US men of comparable age, limiting the ability to generalize our results to men at high cardiovascular risk.

We found a tendency toward a protective effect for higher levels of \( \beta \)-carotene among current and former smokers,

### Table 3. Relative Risk of Myocardial Infarction by Quintiles of Baseline Antioxidant Plasma Levels

<table>
<thead>
<tr>
<th>Quintile of Antioxidant</th>
<th>1 (ref)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>( P ) for Trend†</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-Carotene</td>
<td>0.76</td>
<td>1.11</td>
<td>1.18</td>
<td>1.29</td>
<td>1.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( \beta )-Carotene</td>
<td>0.95</td>
<td>1.07</td>
<td>1.14</td>
<td>1.23</td>
<td>1.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( \beta )-Cryptoxanthin</td>
<td>0.91</td>
<td>1.01</td>
<td>1.11</td>
<td>1.21</td>
<td>1.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.89</td>
<td>1.02</td>
<td>1.10</td>
<td>1.24</td>
<td>1.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.93</td>
<td>1.05</td>
<td>1.15</td>
<td>1.31</td>
<td>1.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Retinol</td>
<td>0.91</td>
<td>1.04</td>
<td>1.12</td>
<td>1.26</td>
<td>1.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( \alpha )-Tocopherol</td>
<td>0.84</td>
<td>1.02</td>
<td>1.12</td>
<td>1.26</td>
<td>1.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( \gamma )-Tocopherol</td>
<td>0.87</td>
<td>1.06</td>
<td>1.20</td>
<td>1.46</td>
<td>1.85</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are OR with 95% CI in parentheses; ref indicates reference category (lowest quintile of baseline antioxidant level).

*Values are median values (ng/mL) for each quintile, based on weighted batch-specific medians of control subjects.

†Tests for trend used median values for the quintiles of the distribution among control subjects.

‡Adjusted for matching variables age and smoking (current, former, never).

§Additionally adjusted for body mass index; total and HDL cholesterol; history of hypertension, diabetes mellitus, and parental MI before age 60; frequency of vigorous exercise (6 categories); alcohol consumption (7 categories); multivitamin use (never, past, current), and assignment to aspirin or \( \beta \)-carotene treatment or placebo.

¶Additionally adjusted for body mass index; total and HDL cholesterol; triglycerides; levels of other tocopherol (quintiles); history of hypertension, diabetes mellitus, and parental MI before age 60; frequency of vigorous exercise (6 categories); alcohol consumption (7 categories), and assignment to aspirin or \( \beta \)-carotene treatment or placebo.

Data available for 257 cases and their matched control subjects.
which is in agreement with previous results. However, the number of current smokers (14.7%) in our study population is small, and, more importantly, levels of β-carotene are lower among smokers. Residual confounding by amount of smoking may therefore have influenced these results. Lutein, contained mostly in dark green vegetables, has recently been suggested to protect against the development of atherosclerosis. However, we found no evidence for a reduced risk of MI among initially healthy men with higher levels of lutein. Furthermore, although lycopene is the predominant plasma carotenoid, exhibiting the strongest antioxidant capacity among the carotenoids in vitro, our findings did not support the previous cross-sectional finding suggesting a protective effect of lycopene on MI.11 Our null finding for lycopene is in agreement with previous results.5 However, the active excretion of lycopene is not surprising because retinol levels are highly regulated and vary little with intake, in contrast to carotenoids and vitamin E.22

Of the two principal forms of vitamin E, γ-tocopherol has the strongest antioxidant capacity and is most prevalent in the diet. In contrast, α-tocopherol predominates in the circulation and is the major form of vitamin E in supplements, which reduce circulating γ-tocopherol levels. Inverse associations between dietary vitamin E and cardiovascular death have been reported, whereas a study from our group observed a reduced risk of CHD associated with supplemental but not dietary vitamin E.12 Data on plasma tocopherols and CHD risk are limited. Our data and prospective results from the Multiple Risk Factor Intervention Trial (MRFIT) study showed no evidence for a protective effect of higher plasma vitamin E levels on MI risk. Unlike plasma α-tocopherol, γ-tocopherol may not reflect dietary vitamin E intake due to the active excretion of γ-tocopherol. Furthermore, tocopherols are strongly associated with lipoproteins, and although we adjusted for these in our analyses, residual confounding by lipoproteins may partly be responsible for the observed association between γ-tocopherol and risk of MI. Unpublished data from the Nurses’ Health Study indicate that dietary trans fat intake is correlated with both dietary γ-tocopherol (r=0.49, P<0.001) and plasma γ-tocopherol levels (r=0.16, P=0.03); in contrast, trans fat intake is inversely correlated with plasma α-tocopherol (r=−0.19, P=0.01). Thus, elevated plasma γ-tocopherol levels might be a marker of trans fat intake. In our current study, we had no information about dietary trans fat intake. This issue should be addressed in studies with more comprehensive dietary information.

Although in our current analyses we found no apparent inverse associations of any of the plasma carotenoids and tocopherols with MI risk (apart from β-carotene among smokers), intake of fruit and vegetables has been shown to protect against CHD.35 Our search for nutrients responsible for this beneficial effect should not keep us from recommending consumption of fruits and vegetables. Current trials testing higher dose of supplemental α-tocopherol will provide important data on their role in primary prevention in low risk populations.

Acknowledgments

This study was supported by National Institutes of Health grants CA42182, CA58684, CA78293, and CA90598; Dr Hak was supported by grants from the Netherlands Organization for Scientific Research and the Foundation De Drie Lichten.

References


Plasma Carotenoids and Tocopherols and Risk of Myocardial Infarction in a Low-Risk Population of US Male Physicians
A. Elisabeth Hak, Meir J. Stampfer, Hannia Campos, Howard D. Sesso, J. Michael Gaziano, Walter Willett and Jing Ma

_Circulation_. 2003;108:802-807; originally published online August 4, 2003;
doi: 10.1161/01.CIR.0000084546.82738.89
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/108/7/802

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/